Confirmation of *Rickettsia conorii* Subspecies *indica* Infection by Next-Generation Sequencing, Shandong, China

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We describe 3 similar cases of rickettsial disease that occurred after tick bites in a mountainous rural area of Shandong Province, China. Next-generation sequencing indicated the etiologic agent of 1 patient was *Rickettsia conorii* subspecies *indica*. This agent may be more widely distributed across China than previously thought.

Shandong is an eastern coastal province of China. Four natural-focal diseases—severe fever with thrombocytopenia syndrome, human granulocytic anaplasmosis, endemic typhus, and scrub typhus—are thought to have the most severe effects on human health in Shandong Province (*1*). However, as in other parts of China, exposure to rickettsial pathogens in eastern provinces is expected because of the prevalence of human-biting ticks (*2*,*3*). Specifically, Japanese spotted fever caused by *Rickettsia japonica* is endemic to Shandong; *R. japonica* and 2 other novel *Rickettsia* spp. were found in the Asian longhorned tick (*Haemaphysalis longicornis*) (*2*). Because rickettsioses have similar clinical manifestations but vary in severity (i.e., incidence of illness and death), laboratory investigation is essential for understanding the epidemiology of tick-borne diseases. We obtained sequences of *Rickettsia conorii* subspecies *indica* (ITTR) infection from 1 case; 2 other cases of spotted fever rickettsiosis (SFGR) with similar epidemiologic history and clinical features were treated at the same hospital (Appendix Table 1, Figure 1, https://wwwnc.cdc.gov/EID/article/27/10/20-4764-App1.pdf). This study was approved by the ethics committee of Qilu Hospital, of Shandong University, Jinan, Shandong, China. All patients signed consent forms.

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The Study

In the summer of 2019, a 53-year-old man (patient 1) was hospitalized with a 5-day history of fever (41°C), influenza-like symptoms, and generalized maculopapular rash (Figure 1, panel A). A farmer working in a rural mountainous area of Zibo, Shandong Province, he was bitten by a tick 6 days before onset of illness. At admission, clinical blood tests revealed elevated leukocyte count (12.91×10^9 cells/L) with neutrophilia (90.5%) and thrombocytopenia (73×10^9/L), as well as increased procalcitonin (3.870 ng/mL) and C-reactive protein (38.31 mg/mL). Rickettsiosis was suspected, and oral minocycline was prescribed on the second day after admission. Symptoms subsided after 2 days of treatment; the patient was discharged from the hospital 6 days later. Serum samples collected on days 8 and 24 after onset of illness tested positive for *Rickettsia conorii* IgG (titers 1,024 at day 8 and 16,384 at day 24) by immunofluorescence assay (IFA) (Fuller Laboratories, http://www.fullerlaboratories.com).

Patient 2, a 41-year-old female agriculture worker from Jinan, the capital of Shandong Province, came from an environment similar to that of patient 1. Patient 2 was hospitalized 18 days after a tick bite; symptoms were an 8-day history of fever (39°C), meningitis, and a sparsely spread purpuric rash (Figure 1, panel B). Intravenous doxycycline treatment was initiated 1 day after admission. Four days after admission, despite 2 days of treatment, the patient experienced seizures, coma, and cardiac arrhythmia. After 2 more days of intravenous doxycycline treatment, the patient improved and was discharged 4

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days later. Serum samples collected on days 9 and 22 after onset of illness tested positive for *R. conorii* IgG by IFA (titers 128 at day 9 and 1,024 at day 22).

Patient 3, a 45-year-old woman, had a history of travel to a farming area in Tai'an, Shandong Province, and was bitten by a tick 8 days before onset of illness. At admission, she had a 5-day history of fever (39°C). She did not have rash but had an ulcerated eschar on her right foot (Figure 1, panel C). Blood tests at hospital admission revealed elevated leukocyte count (10.12×10^9 cells/L), procalcitonin (0.108 ng/mL), and C-reactive protein (46.39 mg/mL). The patient was treated with minocycline beginning the next day after admission; she began to improve on day 3 of treatment and was discharged after 3 more days. Serum samples collected on days 9 and 20 after onset of illness tested positive for *R. conorii* IgG by IFA (titers 64 at day 9 and 1,024 at day 20).

Conventional bacterial cultures of blood samples collected at admission yielded negative results for all 3 patients, as did viral nucleic acid detection of pharyngeal swab samples. Results of serologic ELISA tests for *Coxiella burnetii* phase II IgG (IBL International GmbH, https://www.ibl-international.com), *Rickettsia typhi* IgM (Fuller Laboratories), and *Orientia tsutsugamushi* IgM (InBios International, Inc., https://inbios.com) were all negative.

To identify the potential causative pathogen, we performed next-generation sequencing (NGS) on the Ion Torrent platform (Thermo Fisher Scientific, https://www.thermofisher.com) by using DNA extracted from the peripheral blood of patient 1, collected on day 7 after onset of fever and before administration of antimicrobial drugs. The sequencing data are deposited at the National Center for Biotechnology Information Sequence Read Archive (accession no. SRR10855057). We mapped those sequences to *R. conorii* ITTR (Appendix Figure 2). Coverage was low except for 16S and 23S rRNA genes, but matching sequences were found across the ITTR genome and to other *Rickettsia* genomes (data not shown). We identified reads mapping to specific *Rickettsia* genomes by using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Appendix Table 2). We identified the *Rickettsia*-specific 16S rRNA gene sequences with the Ribosomal Database Project Classifier by using Geneious Prime 19 (Geneious, https://www.geneious.com) (Appendix Table 2). Moreover, we identified sequence reads matching 3 genes commonly used for speciation of *Rickettsia* (*glt*A, *omp*A, *omp*B); 6 other proteins; and 1 pseudogene, *rnp*B, and containing or flanking 20 of the 33 rickettsial tRNAs (33 reads) (Figure 2; Appendix Table 2). Many sequence reads mapped most closely to ITTR or to ITTR and its closest relative, *R. conorii conorii* Malish 7; sequence reads mapped less frequently to the other subspecies, *R. conorii caspia* and *R. conorii israelensis.*

Conclusions

Many tickborne rickettsiae have been described from China, including *R. heilongjiangiensis*, *R. sibirica* BJ-90, *R. sibirica mongolotimonae*, *R. monacensis*, *R. raoultii*, *R. slovaca*, *R. japonica*, *Candidatus* R. tarasevichiae, and other *Rickettsia* spp. of unknown pathogenicity (*2*,*4*). We molecularly confirmed a case of SFGR disease in eastern China caused by *R. conorii* subsp. *indica*. We identified 2 other serologically confirmed cases of SFGR with similar history of tick bite, similar clinical manifestations, and shared epidemiologic features.

NGS technology provided the specific etiology of SFGR in 1 of these patients. The single NGS read length exceeded the size of tRNAs, so they were informative for identification, but diagnostic sites were also obtained for protein fragments (Appendix Table 2). The sensitivity of NGS depends on the type of the clinical sample, the timing of collection, and desirability for depleting human DNA to improve sensitivity of pathogen detection by increasing the number of agent sequences (*5*).

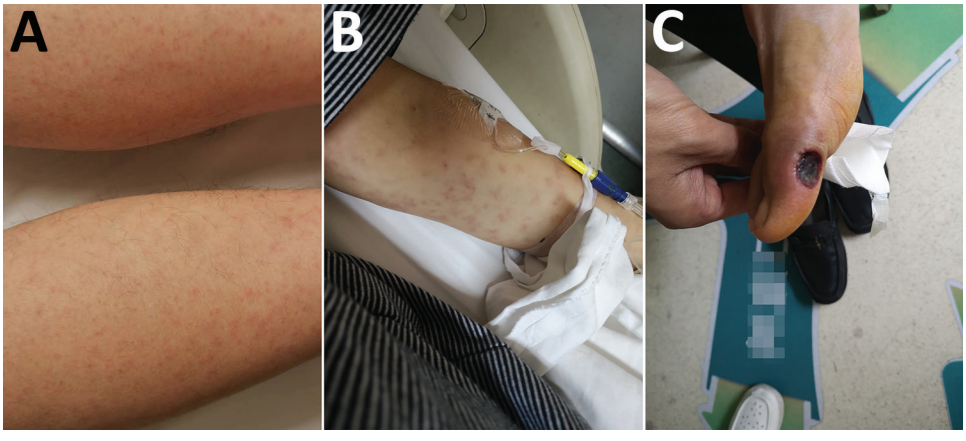

Figure 1. Skin manifestations of patients in study of confirmation of *Rickettsia conorii* subspecies *indica* infection by next-generation sequencing, Shandong, China. A) Rash in patient 1; B) rash in patient 2; C) eschar in patient 3

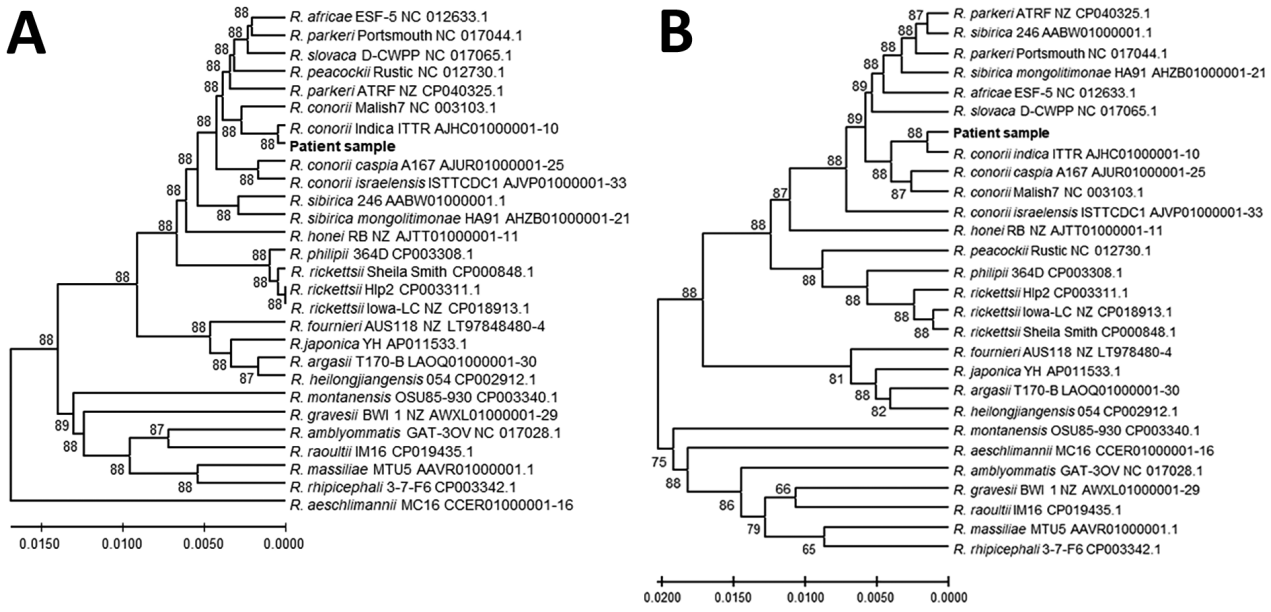

Figure 2. Genetic relationships of the spotted fever group rickettsia detected in blood of patient 1 in study of confirmation of *Rickettsia conorii* subspecies *indica* infection by next-generation sequencing, Shandong, China. This analysis used concatenated sequences from 27 spotted fever rickettsial genomes homologous to the patient sequences (shown in bold text). A) Analysis of 1,379 positions in the tRNA-associated sequences; B) analysis of 1,519 positions in the protein gene–associated sequences. Each tree was constructed upon concatenation of 6 different genome sites (Appendix Table 2, https://wwwnc.cdc.gov/EID/article/27/10/20-4764-App1.pdf); the consensus of reads from sites with overlapping reads was used. The evolutionary relationships were inferred by using UPGMA implemented in MEGA X (*15*). The optimal trees are shown. The percentage of replicate trees in which the taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances computed by using the Kimura 2-parameter method are in the units of the number of base substitutions per site. The proportion of sites where ≥1 unambiguous base is present in ≥1 sequence for each descendent clade is shown next each internal node in the tree. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Scale bars indicate the percentage of nucleotide variation between the sequences.

R. conorii is divided taxonomically into 4 subspecies: *R. conorii conorii*, *R. conorii caspia*, *R. conorii israelensis*, and *R. conorii indica* (*6*). The members of this group exhibit substantial genome sequence similarity and shared antigenic makeup; however, the diseases they cause might be distinguished by specific clinical manifestations, rates of illness or death, and the areas of their endemicity and predominant tick vectors (*6*). PCR-confirmed clinical cases caused by ITTR have been diagnosed in India (*7*), Sicily (*8*) and Xinjiang Uygur Autonomous Region, China (GenBank accession nos. MG190327–9). Well-documented entomologic surveys indicate a broader area of circulation of this etiologic agent, extending beyond India and Pakistan (*9*) to Laos (*10*) and western provinces of China (*11*,*12*). In those areas, ITTR is associated either with *Rhipicephalus turanicus* (sheep tick) or *Rh. sanguineus* (brown dog tick) collected from pet dogs (*12*,*13*), suggesting a high probability of human exposure, given the proximity of these animals to human habitats. Our findings indicate that circulation of ITTR in Shandong Province and transmission to humans occurs in rural mountainous areas where the presence of both tick species has been documented (*3*,*14*). These findings suggest transmission of 1 or several SFGRs to humans might occur across China, thus requiring additional diagnostic and surveillance efforts that could lead to improved identification and management of patients with these infections.

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About the Author

Dr. Xu is an infectious disease doctor in Qilu Hospital of Shandong University. Her research interests include early detection and differential diagnosis of diseases with febrile syndrome. Dr. Gai is working to explore novel pathogen detection methods and products based on NGS technology. He is interested in improving application of next-generation sequencing for routine clinical diagnostics.

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Appendix

Appendix Table 1. Clinical features and laboratory findings in 3 spotted fever patients, Shandong Province, China*

Characteristic, symptom, or laboratory parameter	Reference range	Patient 1	Patient 2	Patient 3
Demographics and exposure history				
Age, y		53	41	45
Sex		M	F	F
History of tick exposure		Yes	Yes	Yes
Time between tick bite and illness onset, d		6	10	8
Time to admission after disease onset, d		5	8	5
Clinical symptoms				
Elevated temperature		Yes (41°C)	Yes (39°C)	Yes (39°C)
Rash		Yes	Yes	No
Headache		Yes	Yes	Yes
Chills		Yes	Yes	Yes
Myalgia		Yes	Yes	Yes
Eschar		No	No	Yes
Nausea		No	Yes	Yes
Vomiting		No	Yes	Yes
Diarrhea		Yes	No	No
Neck stiffness		No	Yes	No
Duration of hospitalization, days		9	10	7
Time to follow-up visit, days		21	23	20
Hematologic test				
Leukocyte count, 10^9/L	3.5–9.5	12.91	5.48	10.12
Neutrophils, %	40–75	90.50	77.20	82.4
Eosinophils, %	0.4–8.0	0.00	0.20	0.40
Hemoglobin, g/L	130–175	151.0	86.0	136
Platelet count, 10^9/L	125–350	73	256	325
Procalcitonin, ng/mL	<0.1	3.870	0.064	0.108
C-reactive protein, mg/L	0–10	38.31	21.0	46.39
Erythrocyte sedimentation rate, mm/h	0–20	29.00	77.0	87
Biochemical test				
Alanine aminotransferase, U/L	21–72	66	19	75
Aspartate aminotransferase, U/L	17–59	62	23	65
Albumin, g/L	35–50	31	41	45
Total bilirubin, μmol/L	3–22	49	16	18
Conjugated bilirubin, μmol/L	0–5	7	0	4
Unconjugated bilirubin, μmol/L	0–19	21	14	18
Sodium, mmol/L	137–145	130	133	138
Calcium, mmol/L	2.1–2.55	2.03	2.23	2.43
Phosphorus, mmol/L	0.81–1.45	0.52	1.16	0.85
Urinalysis				
Erythrocytes (per high-power field)	0–10	329.56	35.20	NA
Blood	–	3+	3+	NA
Bilirubin	–	1+	–	NA.
Protein	–	1+	–	NA
Urobilinogen	–	3+	–	NA
Fecal analysis				
Blood	–	–	–	NA
Cerebrospinal fluid measurements				

Characteristic, symptom, or laboratory parameter	Reference range	Patient 1	Patient 2	Patient 3
Leukocyte count, per mm^3	≤5	NA	1100	NA
Neutrophils, %		NA	76	NA
Lymphocytes, %		NA	18	NA
Glucose, mmol/L	2.5–4.5	NA	2.17	NA
Chlorine, mmol/L	120–130	NA	109	NA
Protein, g/L	0.15–0.45	NA	6.71	NA

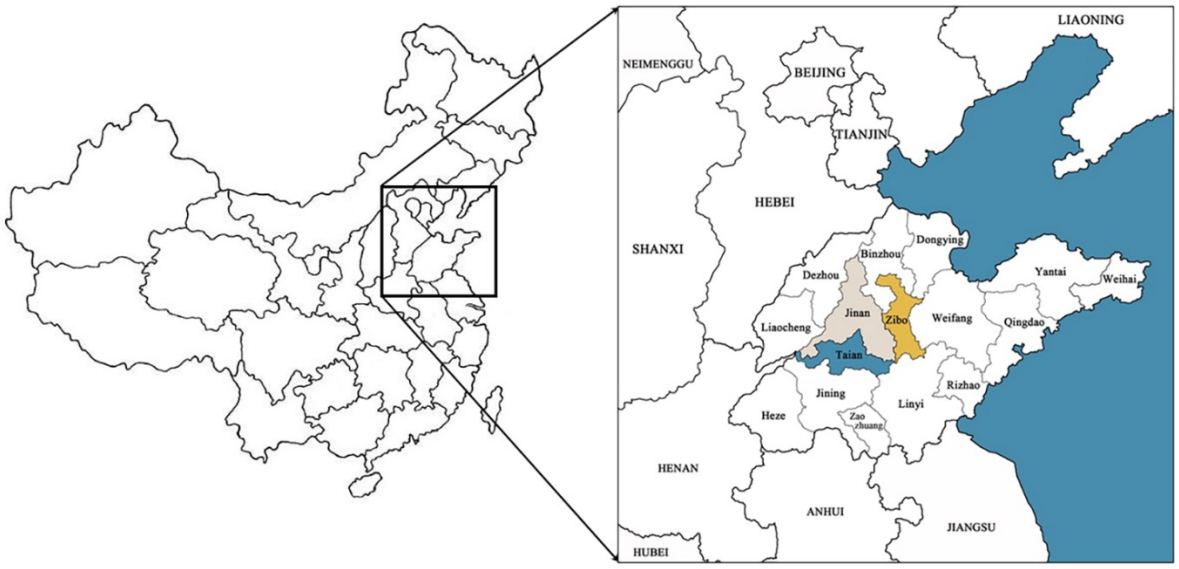
*NA, not available (not performed or not reported); +, positive; –, negative.

Appendix Table 2. Characteristics of Ion Torrent next-generation sequencing reads mapping with high similarity to the *Rickettsia* genome sequencing (complete and partial) available at NCBI Genome BLAST, study of *Rickettsia conorii* subspecies *indica* infection confirmed by next-generation sequencing, Shandong, China

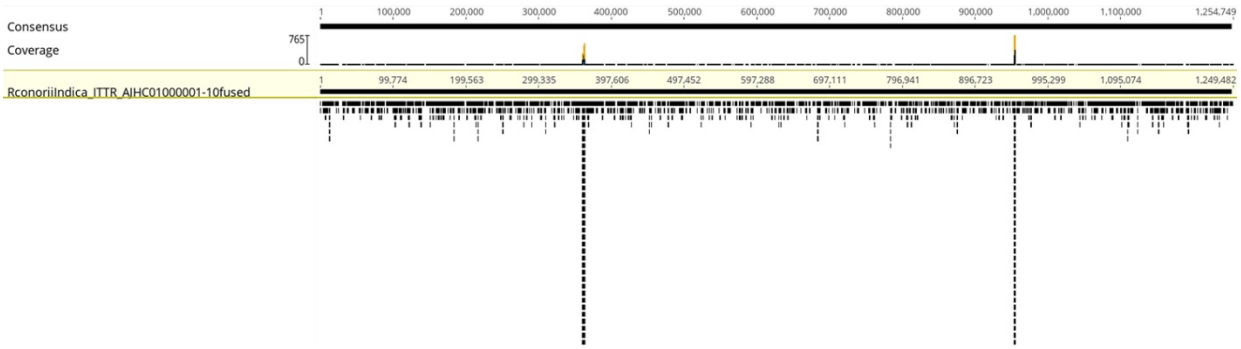
SRA read ID	Read length (nt)	% identity	Gene (chromosome location)	Rickettsia specificity
tRNA associated			(tRNA coverage/total)*	
SRR10855057.3634361.1	233	99.15	tRNA-Ala-GGC (45/76)	ITTR>R. conorii (Caspia, ISTT, Malish), other Rickettsia
SRR10855057.1770868.1	150	98.00	tRNA-Arg-TCT (77/77)	ITTR = Malish = Tenjiku01 = Rickettsia endosymb Proechinophthirus>other Rickettsia
SRR10855057.3222332.1	182	100.00	tRNA-Asn-GTT (5′ flank)	Many Rickettsiae, R. conorii (Caspia, ITTR, Malish) >R. conorii (ISTT)
SRR10855057.2512399.1	249	99.60	tRNA-Cys-GCA (75/75)	ITTR = R. parkeri, R. sibirica mong.>R. conorii (3), other Rickettsia
SRR10855057.2413080.1	184	98.92	tRNA-Gln-TTG (15/74)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.3506905.1	240	98.17	tRNA-Glu-TTC (52/75)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.2889951.1	239	98.76	tRNA-Glu-TTC (75/75)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.882327.1	228	100.00	tRNA-His-GTG (77/77)	ITTR = Malish>R. conorii (ISTT, Caspia), other Rickettsia
SRR10855057.3412805.1	115	97.41	tRNA-His-GTG (14/77)	ITTR = Malish other Rickettsia>R. conorii (Caspia, ISTT)
SRR10855057.819370.1	150	99.34	tRNA-His-GTG (11/77)	ITTR = Malish = other Rickettsia>R. conorii (Caspia, ISTT)
SRR10855057.1459281.1	226	98.66	tRNA-Ile-GAT (76/77)	ITTR>R. conorii (Malish, ISTT, Caspia), other Rickettsia
SRR10855057.2012331.1	248	100.00	tRNA-Leu-GAG (5′ flank)	ITTR = Malish = Caspia>R. conorii ISTT, other Rickettsia
SRR10855057.1580324.1	216	97.72	tRNA-Leu-TAA (86/86)	ITTR = Malish = R. parkeri = R. sibirica mong.>R. conorii (ISTT, Caspia), other Rickettsia
SRR10855057.3515495.1	243	99.59	tRNA-Leu-TAA (80/86)	ITTR = Malish>R. conorii (ISTT, Caspia), other Rickettsia
SRR10855057.1645045.1	240	99.17	tRNA-Leu-TAA (3′ flank)	ITTR>R. conorii (Malish, ISTT, Caspia), other Rickettsia
SRR10855057.2952780.1	160	100.00	tRNA-Leu-TAA (3′ flank)	ITTR>Malish = R. parkeri = R. slovaca
SRR10855057.529019.1	227	98.68	tRNA-Lys-TTT (76/76)	ITTR>R. conorii (Malish, Caspia, ISTT), other Rickettsia
SRR10855057.796844.1	264	100.00	tRNA-Phe-GAA (3′ flank)	R. conorii (Malish, Caspia, ITTR)>R. conorii ISTT, other Rickettsia
SRR10855057.2538618.1	108	99.07	tRNA-Phe-GAA (24/76)	R. conorii (Malish, Caspia, ITTR), R. parkeri, R.sibirica>R. conorii ISTT, other Rickettsia
SRR10855057.2148777.1	208	99.04	tRNA-Pro-TGG (5′ flank)	ITTR = Malish>R. conorii (ISTT, Caspia), other Rickettsia
SRR10855057.1901026.1	210	99.52	tRNA-Pro-TGG (5′ flank)	ITTR = Malish>R. conorii (ISTT, Caspia), other Rickettsia
SRR10855057.2442098.1	119	93.39	tRNA-Pro-TGG (8/77)	Many Rickettsia
SRR10855057.3656176.1	135	96.92	tRNA-Pro-TGG (62/77)	Many Rickettsia
SRR10855057.2888707.1	98	96.88	tRNA-Pro-TGG (73/77)	Many Rickettsia
SRR10855057.650629.1	254	98.45	tRNA-Ser-GCT (3′ flank) (h prot)	ITTR = R. slovaca, R. africae>R. conorii (Malish, Caspia, ISTT) other Rickettsia
SRR10855057.108521.1	156	100.00	tRNA-Ser-GGA (37/88)	ITTR>R. conorii (Malish, Caspia, ISTT), other Rickettsia
SRR10855057.1718882.1	176	100.00	tRNA-Ser-GGA (88/88)	ITTR>R. conorii (Malish, Caspia, ISTT), other Rickettsia
SRR10855057.1545323.1	241	98.77	tRNA-Ser-TGA (89/90)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.2148249.1	222	100.00	tRNA-Thr-CGT (5′ flank) (bamA)	ITTR>R. conorii (Malish, Caspia, ISTT), other Rickettsia
SRR10855057.3442561.1	223	99.55	tRNA-Thr-CGT (5′ flank) (bamA)	ITTR>R. conorii (Malish, Caspia, ISTT), other Rickettsia
SRR10855057.850105.1	221	98.66	tRNA-Thr-TGT (10/75)	ITTR = Malish, R. peacockii>other Rickettsia
SRR10855057.3110358.1	248	97.64	tRNA-Val-GAC (71/77)	ITTR = Malish>other Rickettsia, R. conorii (ISTT, Caspia)
RNA subunit (M1 RNA) of ribonuclease P, rnpB				
SRR10855057.2420743.1	164	100.00	rnpB	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
16S ribosomal RNA			16S rRNA (rrs)	
SRR10855057.206203.1	226	100.00	rrs	Many Rickettsia including R. conorii (Malish, ITTR, Caspia, ISTT)
SRR10855057.991444.1	212	99.53	rrs	Many Rickettsia including R. conorii (Malish, ITTR, Caspia, ISTT)
SRR10855057.1633641.1	150	96.23	rrs	R. canadensis>other Rickettsia
SRR10855057.1948617.1	106	96.23	rrs	Bemisia Rickettsia, R. bellii>other Rickettsia
SRR10855057.2305806.1	180	99.42	rrs	Many Rickettsia including R. conorii (Malish, ITTR, Caspia, ISTT)
SRR10855057.3297795.1	210	99.30	rrs	Many Rickettsia including R. conorii (Malish, ITTR, Caspia, ISTT)

SRA read ID	Read length (nt)	% identity	Gene (chromosome location)	Rickettsia specificity
SRR10855057.3401137.1	200	99.50	rrs	Many Rickettsia including R. conorii (Malish, ITTR, Caspia, ISTT)
23S ribosomal RNA			23S rRNA (rrl)	
No reads mapping				No matches found
Citrate synthase			gltA	
SRR10855057.1205776.1	228	100.00	gltA	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.2326332.1	182	100.00	gltA	R. conorii (ITTR = Malish = Caspia = ISTT), other Rickettsia
SRR10855057.3271726.1	229	100.00	gltA&3′ flank	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
Outer membrane protein OmpB			ompB (sca5)	
SRR10855057.3323415.1	204	97.58	ompB (sca5) and 5′ flank	ITTR>R. conorii (Caspia, ISTT, Malish), other Rickettsia
SRR10855057.1268928.1	231	100.00	ompB (sca5)	ITTR = Malish>other Rickettsia
SRR10855057.436084.1	213	96.36	ompB (sca5)	ITTR = Malish>other Rickettsia
SRR10855057.1634014.1	222	98.66	ompB (sca5)	ITTR>R. conorii (Caspia, ISTT, Malish), other Rickettsia
SRR10855057.2155308.1	155	100.00	ompB (sca5)	ITTR = Malish>other Rickettsia
SRR10855057.2143700.1	223	100.00	ompB (sca5)	ITTR = Malish>other Rickettsia
SRR10855057.3503391.1	217	99.54	ompB (sca5)	ITTR>R. conorii (Caspia, ISTT, Malish), other Rickettsia
Outer membrane protein OmpA			ompA (sca0)	
SRR10855057.1793457.1	232	100.00	ompA (sca0) and 5′flank	ITTR>R. conorii (Malish, Caspia, ISTT), other Rickettsia
SRR10855057.1750933.1	232	95.34	ompA (sca0)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.1660860.1	147	95.24	ompA (sca0)	R. conorii (ITTR = Malish = ISTT = Caspia) >other Rickettsia
SRR10855057.810562.1	262	100.00	ompA (sca0)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.790884.1	245	100.00	ompA (sca0)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.611967.1	200	99.50	ompA (sca0)	ITTR = Malish>R. conorii (Caspia, ISTT), other Rickettsia
SRR10855057.249559.1	200	94.76	ompA (sca0)	R. conorii (Caspia, Malish, ITTR, ISTT) = R. africae = R. parkeri = R. sibirica>other Rickettsia
Cell surface antigen 4			sca4	
No reads mapping				
Pseudogene RC_RS06985-IGS				
SRR10855057.210346.1	233	99.57	RC_RS06985 (3′ flank)	ITTR>R. conorii (Malish, ISTT, Caspia), other Rickettsia
SRR10855057.1977290.1	183	99.46	RC_RS06985 (3′ flank)	ITTR>R. conorii (Malish, ISTT, Caspia), other Rickettsia
SRR10855057.3088753.1	183	98.92	RC_RS06985 (3′ flank)	ITTR>R. conorii (Malish, ISTT, Caspia), other Rickettsia
SRR10855057.3018756.1	176	100.00	RC_RS06985 (3′ flank)	ITTR>R. conorii (Malish, ISTT, Caspia), other Rickettsia
UDP-3-O-(3-hydroxymyristoyl)glucosamine N-acyltransferase (luxD) RC_RS00050 (5′ flank)				
SRR10855057.3390182.1	210	100.00	lpxD (5′ flank)	ITTR = Malish>R. conorii (ISTT, Caspia), other Rickettsia
IGS of AsmA family protein RC_RS02405 and rimM RC_RS02410				
SRR10855057.2154203.1	242	99.59	asmA-rimM IGS	ITTR = Malish>R. conorii (ISTT, Caspia), other Rickettsia
Preprotein translocase subunit SecG RC_RS00570 (5′ flank) (tRNA-Thr-GGT)(3‘ flank)				
SRR10855057.65175.1	150	96.67	secG (5′ flank)	ITTR = Malish = R. parkeri, R. sibirica = R. slovaca>other Rickettsi
Rod shape-determining protein MreC RC_RS05910 (3′ flank)				
SRR10855057.870441.1	151	95.36	mreC (3′ flank)	ITTR>R. conorii (Malish, Caspia, ISTT), other Rickettsia

*Where indicated, numbers in brackets correspond to the total nucleotide length of the tRNA gene (denominator) and the number of tRNA nucleotides overlapping with a listed next-generation sequencing read (numerator). IGS = intergenic spacer. Reads providing specific identification of *Rickettsia conorii* subspecies *indica* infection are shown in gray. ITTR, *R. conorii indica*; Caspia, *R. conorii caspia*; ISTT, *R. conorii israelensis*; Malish, *R. conorii conorii* strain Malish.



Appendix Figure 1. Map of the geographic locations where exposure to rickettsial pathogens occurred, Shandong Province, China.



Appendix Figure 2. Mapping by Geneious Prime BLAST of 9581 Ion Torrent reads to the *Rickettsia conorii indica* genome (AJHC01000001.1–10.1) including 10 fused contigs which are syntenic to the complete *R. conorii conorii* Malish 7 genome (NC_003103.1). The large pileups of 16S rRNA (coordinates 359040–361800; 3,991 reads) and 23S rRNA (coordinates 950294–951801; 2,641 reads) gene reads are truncated in the figure.